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Research Note

Cellulase Inhibition by Polyphenols in Olive Fruits

ABSTRACT

The inhibition of cellulolytic enzymes by polyphenolic compounds present in the olive fruit (Hojiblanca variety) and by those which remain in the acetone powder is described. Basidiomycetes cellulase and cellulolytic enzyme extracts from the fruit itself were tested. An inhibition of 80% was reached. Polyphenols were determined by derivative spectroscopy.

INTRODUCTION

Certain properties of plants, such as nutritional value (Griffiths, 1981), protection against microorganisms (Lund, 1983), and taste (Vazquez *et al.*, 1971), can be attributed to the presence of polyphenols, being clearly influenced by the capacity of forming cross-linkages with proteins, which could lead to the precipitation of soluble proteins and/or an inhibition of enzymic systems (Meetche *et al.*, 1980). There is a close relationship between the concentration of polyphenols from diverse natural products such as berries, pea pods, beans, leaves of different types of plants, and the inhibition of cellulases and other types of enzymes, such as pectinases, amylases, and trypsin (Bell *et al.*, 1965; Griffiths, 1981; Ozawa *et al.*, 1987).

Olive flesh contains polyphenols, up to 7% of dry weight, expressed as caffeic acid (Vazquez *et al.*, 1971). The work presented here studies the inhibitory effect of olive polyphenols on Basidiomycetes cellulase and on the cellulolytic enzymes from the olives themselves, which may have some influence on cell-wall structure modifications that take place during ripening and softening of the fruit.

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MATERIAL AND METHODS

Olive samples were of the Hojiblanca variety (*Olea europaea arolensis*) from the Experimental Agricultural Station in Cordoba (Spain). The fruits were frozen and kept at −20°C until used. The enzymes used were: (a) cellulase from Basidiomycetes (E.C.3.2.1.4.) (Merck) dissolved in phosphate buffer, 0·02M, pH 6·6, to a concentration of 5 mg/ml; (b) enzymic extract of olives. Olive flesh (25 g) was homogenized with acetone (250 ml) at −20°C, using a high speed Sorvall Omnimixer. The homogenized mixture was filtered and the residue of acetone powder washed with cold acetone (3 × 125 ml). The acetone powder was treated at 2·5°C with 0·02M phosphate buffer, pH 6·6 (50 ml) for 2 h, centrifuged at 3000 *g* for 10 min at 1°C (RC-5 Superspeed Refrigerated Centrifuge, SS-34 rotor, Sorvall) and filtered (Millipore SMWP 047005 μ filter). The filtrate was used for the cellulase assay.

Cellulase activity was assayed as described by Hasegawa and Smolensky (1971). Relative units (*B*) of enzymic activity as described by Almin *et al.* (1967) were used.

Extraction and determination of polyphenols

Olive flesh (20 g) was homogenized in ethanol (40 ml) in a Sorvall Omnimixer for 1 min at maximum speed. The rod and container were then washed with ethanol (3 × 20 ml) and the washing liquids were added to the homogenate. The mixture was boiled for 5 min, in order to deactivate enzymes and to extract the polyphenols. Distilled water (400 ml) was added and boiling continued for 1 h. The liquid evaporated during boiling was replaced with water, making up to a final volume of 500 ml; 150–200 ml was filtered and and aliquot taken for quantitative determinations by means of ultraviolet derivative spectroscopy (Vazquez *et al.*, 1971). The spectrum of the second derivative (*D*) was followed according to Montaño *et al.* (1985). Pyrocatechol was used as standard. A UV/Vis Perkin-Elmer Spectrophotometer, model 550 SE, was used for the determination.

RESULTS AND DISCUSSION

In order to establish the inhibitory effect of the polyphenols in olives, the total polyphenols extracted from the fruit as well as those that remain in the enzymatic extract obtained from the acetone powder were used. Basidiomycetes cellulase and enzymic extracts of the fruit were used as enzymes.

Table 1 shows the results obtained by adding an aqueous extract of polyphenols from the olive flesh (that contains 3·30 mg of total polyphenols,

TABLE 1
Inhibition of Cellulase Activity of (a) basidiomycetes Cellulase, (b) Enzymic Extract from Olive Fruit by Total Polyphenols from Olives

Polyphenols solution (ml)[a]	*Cellulase relative units B/h*					*Inhibition (%)*
	Basidiomycetes		*Inhibition (%)*	*Olive fruit*		
	Without inhibitor	*With inhibitor*		*Without inhibitor*	*With inhibitor*	
2	66 887 ± 975	13 131 ± 128	80·4	67 590 ± 789	12 230 ± 97	82·0
5	59 907 ± 850	12 267 ± 54	81·2	58 470 ± 695	9 300 ± 72	84·1
7	56 267 ± 628	8 640 ± 79	84·7	55 217 ± 584	7 530 ± 80	86·4
10	47 663 ± 500	5 687 ± 26	88·0	48 650 ± 327	5 720 ± 54	88·3

The results represent the means of four experiments ± SD.
[a] 8·0 mg of total polyphenols expressed as mg of pyrocatechol/ml solution, obtained from 120 g fresh flesh.

expressed as mg of pyrocatechol/g of fresh flesh) to a Basidiomycetes cellulase and to an enzymic extract from the olive fruit itself, and carrying out the incubation with carboxymethylcellulose, observing inhibitory effect values up to 88%.

In order to study the inhibiting capacity of the polyphenols that remain in the enzymic extracts, two extracts were taken, prepared from acetone powder from green and black olives. Their polyphenol content is shown in

TABLE 2
Inhibitory Effect of Polyphenols from Acetone Powder from Green and Black Olives on Basidiomycetes Cellulase

Polyphenols solution (ml)[a]	*Cellulase activity*[b]		*Inhibition (%)*
	Without inhibitor	*With inhibitor*	
2-G	65 016 ± 873	49 860 ± 506	23·3
2-B	65 016 ± 873	45 000 ± 467	30·8
5-G	58 012 ± 427	36 961 ± 470	36·5
5-B	58 012 ± 427	33 678 ± 380	42·1
7-G	51 876 ± 512	30 592 ± 392	41·5
7-B	51 876 ± 512	27 475 ± 287	47·0

G, green olive; B, black olive.
[a] Green olive: 1·94 mg of total polyphenols expressed as mg of pyrocatechol/ml solution. Black olive: 3·50 mg of total polyphenols, expressed as mg of pyrocatechol/ml solution.
[b] Relative units B/h.

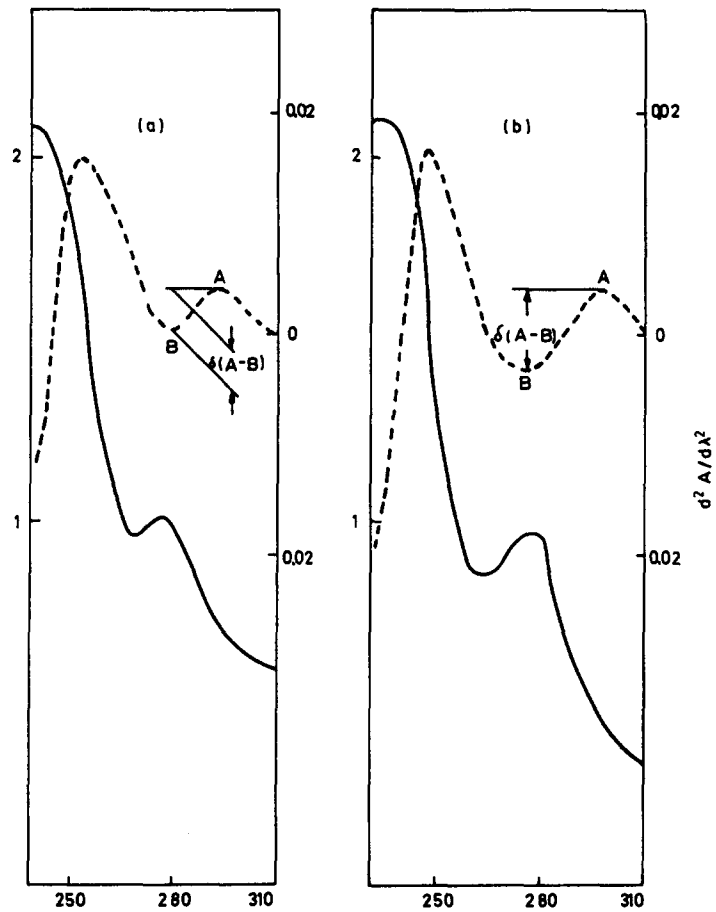

Fig. 1. A normal spectrum and one of the second derivative of extracts originating from (a) green olives and (b) black olives. Calibrate curve: $\delta = 0{\cdot}515c$ ($r = 0{\cdot}9998$) (Montaño *et al.*, 1985). Normal spectrum, sweeping 310–190 nm. Second derivative, ord 0·05. Resp 10, register speed 60 nm/min.

Fig. 1 (a and b), in which the UV absorption spectra show a slight inflection at 278 nm and the spectra of the second derivative, a minimum at 274 nm. The marked inhibiting effect that they exercise on Basidiomycetes cellulase is shown in Table 2, with values up to 47%.

The present study shows how aqueous extracts from polyphenols in olives exercise a marked inhibiting effect on Basidiomycetes cellulase and on cellulases from the olives themselves.

Quantititative measurements of these polyphenols have been made following a new, quick and direct method in which the possible interference of other components of the sample are negligible (Montaño *et al.*, 1985).

As the acetone insoluble powder does not contain the total polyphenols,

since part of these are eliminated in the acetone washings, the real inhibitory effect in the fruits (Table 1) is greater than that found in the acetone powder (Table 2). Comparing the polyphenols contained in 7-G (24·5 mg) (Table 2) with those found in 2 ml fresh flesh (16 mg) (Table 1), it can be seen that the inhibitory effect of 7-G is less, indicating that a considerable part of the polyphenols with the greatest inhibitory effect have been eliminated with the acetone. Consequently, the inhibition depends on the type of polyphenols present in the extracts and this indicates the direction for future research.

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